De Coombs consumption test met geisoleerde kippenerythrocytenkernen in de LE-serologie

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SUMMARY

This thesis gives results of an investigation on the value of the Coombs consumption test in LE serology, more special with isolated chicken red cell nuclei as the antigen.

Chapter I reviews the literature concerning investigations that have allowed some insight in the immunological explanation of the LE cell phenomenon and the reaction of LE serum with nuclei and nuclear components. There is accumulating evidence that the factor responsible for the LE cell phenomenon is an antibody against deoxyribonucleoprotein in which both deoxyribonucleic acid and histone are necessary for the reaction. Moreover in several LE sera separate antibodies against deoxyribonucleic acid, histone and a still unknown antigen in whole nuclei have been detected, which are however not likely to be related to the LE cell phenomenon itself.

Chapter II gives a detailed view on LE serology. The methods that have been used in this field are discussed. It appears that simple methods are comparatively insensitive, while sensitive methods are comparatively complicated. Furthermore it is sometimes uncertain which factor (factors) is (are) demonstrated. Best related to a positive LE cell phenomenon seems to be the Coombs consumption test.

Chapter III deals with general problems in using the Coombs consumption test as a serological method.

Chapter IV first holds technical details on the preparation of isolated chicken red cell nuclei adapted to use in the Coombs consumption test. In this respect previous aging of the chicken red cells and substitution of physiological saline by 0,8 % Tween-80 in Michaelis' buffer P_h 7,42 proved to be of great value. By addition of 0,2 % formol to this solution a washing-liquid was obtained that ensured a steady and homogeneous suspension of the isolated chicken nuclei after having incubated them in human serum.

In our hands Steffens' procedure for the Coombs consumption test proved to be the most accurate and reliable one. Using isolated chicken red cell nuclei as the antigen several aspects of this serological method were investigated. From this the following concept was drawn up:

1. The amount of specific gamma globulin bound per individual nucleus seems to be determined by the strength of the LE serum.
2. The number of nuclei required to demonstrate this in the Coombs consumption test seems to be determined by the sensitivity of the indicator: Coombs serum + sensitized erythrocytes.

Since a number of at least 250 x 10^6 nuclei per ml were required to obtain optimal differences between LE serum and

normal serum, it was concluded that the absolute sensitivity of the Coombs consumption test is poor. This does not necessarily mean that the same is true for the relative sensitivity of the test.

Isolated chicken red cell nuclei proved to adsorb a comparatively large amount of aspecific gamma globulin so that many different washings were required. Nevertheless this gamma globulin could not be completely removed. It was necessary to compare results with normal serum. Since however different normal sera gave different results in so far that the uptake of aspecific gamma globulin varied somewhat, we compared with a known normal serum (standard serum).

For general purposes a suspension of 500 x 10^6 nuclei per ml was used. One ml of this suspension was incubated for half an hour at 37° C with one ml of inactivated serum and after twelve subsequent washings brought into contact with Coombs serum according to Steffen. To obtain comparable results always the same Coombs serum was used. The subsequent loss of titer was determined with a 2 - 4 per cent suspension of sensitized Rh positive erythrocytes. In this way differences between LE serum and standard serum varied from 0 to 7 titers. Although in our experience a difference of one half titer is inconclusive the repeated finding of such a difference was suggestive for a weakly positive reaction. Since however not all sera were repeatedly examined a limit of one titer was established for a positive reaction.

Chapter V summarizes the results obtained with the sera from 264 different individuals. In 74 cases this concerned patients with a positive LE cell phenomenon of whom 47 were suffering from disseminated lupus erythematosus and 23 from rheumatoid arthritis (tabel XXIII). In the first group the mean result numbered 3 titers and in the second group $1\frac{1}{2}$ titers (tabel XXIX, figures 4 and 5). In both groups however several individual exceptions were noted. It might be possible that seriousness of the clinical picture or effect of therapy (steroids) accounts for this.

In the group of 47 LE sera 45 were positive and 2 negative and in the group of 23 RA sera (positive LE cell phenomenon) 13 were positive and 10 negative. Control sera from 65 patients suffering from a wide variety of diseases with negative LE cell phenomenon were usually negative (tabel XXIV). In 7 patients however a weakly positive reaction was noted. In one of these patients formerly a positive LE cell phenomenon had been found. In the other six patients the following diagnoses had been made: periarteritis nodosa, undefined collagen disease, drug allergy, rheumatoid arthritis, ankylosing spondylitis and chronic discoid lupus erythematosus. Sera from 66 healthy donors of the bloodtransfusion service and 59 pregnant women were negative.

In 28 unselected sera from patients with a positive LE cell phenomenon no correlation was found between the number of LE cells per 1000 leucocytes[1] and the quantitative result of the Coombs consumption test (figure 1). In an investigation to find out the reason for this 24 out of these sera were examined[1] for complement fixing antibodies against isolated nuclei, deoxyribonucleoprotein, deoxyribonucleic acid and histone. In 11 sera such antibodies could not be demonstrated. In these cases the number of LE cells again was plotted against the result of the Coombs consumption test (figure 2). Now a significant correlation was found ($p = 0,05$). It was concluded that in addition to LE factor other antinuclear antibodies may influence the result of the Coombs consumption test. In some cases this may account for the discrepancy between a low number of LE cells and a strongly positive Coombs consumption test, in other cases however mere blocking of the LE cell phenomenon seems to be responsible for this.

Twenty selected LE sera were examined by the Schultz-Dale technique for antibodies against deoxyribonucleoprotein, deoxyribonucleic acid, ribonucleic acid and histone. No significant correlation was found between the degree of contraction of strips of intestine sensitized by deoxyribonucleoprotein and the quantitative result of the Coombs consumption test (figure 3). It is however not certain that both techniques are completely comparable in this way.

Chapter VI compares the results with the literature. It is concluded that the Coombs consumption test is a useful method in LE serology, sometimes more sometimes less sensitive than the LE cell phenomenon.

1) Performed by Dr W. Hijmans of Leiden.